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The Activation of Animal Ribosomes by Polyuridylic Acid

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The observation by Nirenberg *et al.*¹ of a polyuridylic acid (poly U) dependent incorporation of phenylalanine into protein by *E. coli* ribosomes has recently been confirmed also by use of ribosomes of animal origin²⁻⁵. Little is yet known, however, about the mechanism of the ribosome-poly U interaction.

Ribosomes from Ehrlich ascitic tumor cells were pre-treated with poly U (0.5 mg/ml) under varied conditions. They were then washed by centrifugation through a layer of plain medium with higher density (sucrose concentration)⁴. When these pre-treated and purified ribosomes were incubated in a system for phenylalanine incorporation, their incorporation activity was significantly higher than that of control particles pre-incubated in the absence of poly U (*cf.* Ref. ⁴). Very soon, however, the poly U induced incorporation activity declined, and the activity of the poly U-treated particles approached that of the untreated ones. This rapid decline was observed even in the absence of energy supply, *i.e.* under conditions where no amino acid incorporation takes place. Addition of poly U restored the incorporation activity.

By utilizing the first few minutes of incubation, when the poly U-dependent incorporation

activity was still relatively high, the effects of varied conditions of pre-incubation were studied in some detail. It was observed that the addition of ATP, ATP-generating system and the soluble fraction of the cytoplasm were without significant influence on the efficiency of the poly U attachment, as estimated from the specific incorporation activity after gradient centrifugation. The binding occurred very rapidly, and no distinct time dependence could be demonstrated. The reaction was inhibited by simultaneously added polyadenylic acid. Increased ionic strength (KCl) or Mg^{2+} concentration in the medium during gradient centrifugation did not markedly influence the extent to which the poly U induced incorporation activity was retained by the particles.

It is concluded that the artificial programming of animal ribosomes with poly U *in vitro* can be relatively efficient. It is considerably less permanent, however, than the programming with informative RNA taking place under physiological conditions. In the latter case a more elaborate mechanism of attachment may be inferred.

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